Stability of Trehalose, Sucrose and Glucose to Nonenzymatic Browning in Mod l Syst ms

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ABSTRACT

The rate of nonenzymatic browning was compared in freeze-dried model systems containing lysine and glucose, sucrose or trehalose at pH 2.5 and a water activity of 0.33. Rate constants for brown color formation (A_{420}) were from 200 to 2000-fold greater for the sucrose than for the trehalose system. Some rate constants for sucrose browning approached those for glucose, indicating that extensive hydrolysis had occurred. The results indicate that use of trehalose rather than sucrose may be of value in stabilizing some systems vulnerable to nonenzymatic browning.

Key Words: nonenzymatic browning, trehalose, sucrose, glucose, amorphous systems.

INTRODUCTION

THE STABILITY of dehydrated food and biological systems is frequently determined by the properties of glasses formed from components such as sugars and polyols. Glass-forming components include sorbitol, fructose, glucose, sucrose and trehalose (Burke, 1986). The preservation of the biological function of proteins in dried formulations using glass technology has been increasingly applied commercially for high-value pharmaceutical proteins (Franks, 1994; Fox, 1995). The glassy state also appears to be important in the stabilization of bacterial spores (Sapru and Labuza, 1993), the survival of dehydrated plant seeds (Williams and Leopold, 1989) and the cryoprotection of some insects (Wasylyk et al., 1988). Some cryptobiotic plants and strains of yeast resistant to dehydration accumulate high amounts of trehalose and the sugar may help preserve the functionality of molecules in such systems (Crowe et al., 1983; Panek et al., 1986). The incorporation of trehalose has been advocated for preserving food quality and biological function during dehydration (Roser, 1991).

Nonenzymatic browning reactions involving amino groups (e.g. ε-NH_2 groups of lysine residues and α-NH_2 groups of proteins) and reducing sugars are an important cause of functionality loss in stored proteins (Brown et al., 1990; van Boekel and Hoenders, 1990; Cerami, 1994). Nonenzymatic browning may have unacceptable nutritional and sensory effects in some stored food products and may be a limiting factor in the shelf life of some products (O'Brien and Morrissey, 1989; O'Brien, 1995). Factors that promote nonenzymatic browning in stored food and biological systems might be detrimental to the functional integrity of amino compounds and could limit the nutritional value and the sensory acceptability of some products. The use of sucrose or trehalose is likely to be an advantage in this respect since they are nonreducing disaccharides (Fig. 1) and would not undergo nonenzymatic browning.

Sucrose may be hydrolyzed during freezing, dehydration and storage (Karel and Labuza, 1968; Schoebel et al., 1969). Such hydrolysis reactions may be promoted by an increase in H^+ concentration that may accompany dehydration (Bell and Labuza, 1992). Flink (1983) showed that browning (A_{400}) and the formation of colorless intermediates such as 5-hydroxymethylfurfural (5-HMF) (A_{280}) in freeze-dried sucrose systems was inversely proportional to initial pH in the pH range 2-5. The relative lability of sucrose in acidic systems was highlighted by the work of Lee and Nagy (1990), who reported that the rate f formation of 5-HMF from sucrose in model systems at pH 3.5 was greater than that from glucose.

No systematic study has been reported to evaluate the stability of trehalose during dehydration compared with sucrose although trehalose might be more stable due to a lower disaccharide bond energy (Roser, 1991). Furthermore, the effects of free amino groups in catalyzing disaccharide hydrolysis are unclear although an influence might be expected on the basis of a mass action effect resulting from the consumption of free reducing sugars in nonenzymatic browning reactions. The objective of this study was to compare the stability of freeze-dried systems containing sucrose or trehalose to nonenzymatic browning in the presence of an amino compound at low pH.

MATERIALS & METHODS

Materials

Crystalline D-(+)-trehalose was obtained from Sigma Chemical Co. (Poole, UK). Sucrose ('Analar' grade) and potassium hydroxide ('Analar' grade) were obtained from Merck Ltd (Poole, UK). Crystalline lysine monohydrochloride, anhydrous crystalline citric acid, anhydrous D-(+)-glucose (ACS reagent), magnesium chloride hexahydrate and phosphorus pentoxide (ACS reagent) were obtained from Sigma Chemical Co. (Poole, UK). Glass Bijou bottles (7 mL capacity) were obtained from Fisons Scientific Equipment, Loughborough, UK.

Methods

Test solutions were prepared in bulk as follows: sugar (sucrose, trehalose or glucose), 2M; lysine, 0.2M; citric acid, 0.5M. Solutions were adjusted to pH 2.5 by dropwise addition of 5N KOH while stirring, using a WPA CD720 pH meter previously calibrated using a 2-point procedure employing buffers at pH 7.0 and 4.0. Following pH adjustment of the bulk solution, aliquots (2 mL) were placed in 7 mL Bijou bottles and frozen as a wedge (exposing maximum surface area) in a chest freezer at −35°C for 48 hr. Samples were subsequently stored over dry ice for 4 hr as described by Roos and Karel (1991) prior to freeze drying in a Virtis Freezemobile 6 freeze drier (Supplied by Techmation Ltd, London) over a 48hr period. Chamber pressure was maintained at 1.1 × 10^{-3} Pa during the drying cycle. Freeze dried samples were further dehydrated in a vacuum desiccator over phosphorus pentoxide for 5 days followed by 72 hr over a saturated solution of $MgCl_2$ to give an a_ f 0.33 (Greenspan, 1977).

Moisture contents were determined gravimetrically by measuring the weight loss upon drying at 60°C *in vacuo* for 72 hr. Values for glass transition temperature, T_g, were determined by differential scanning calorimetry using a Perkin-Elmer 7 series Thermal Analysis System at a scan rate of 10°C/min. Glass transition temperatures were measured as the onset temperature for the first derivative of the baseline shift f the DSC thermogram. Crystallization in the trehalose system was assessed using ^{13}C-NMR spectroscopy (300 scans) on a Bruker MSL300 spectrometer fitted with a CP-MAS probe.

Equilibrated samples were hermetically sealed with teflon rubber septa and placed in vens at 40°C, 60°C or 90°C for appropriate incubation times. Preliminary experiments were used to optimize incubation times. Duplicate samples were used at all time points. Samples were removed at 4, 12, 24, 48, 80, 130, 178, and 218 hr at 40°C; additional samples were taken at 6 and 34 hr at 60°C. Samples of the heated glucose and sucrose systems were withdrawn at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and

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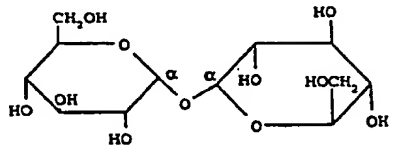
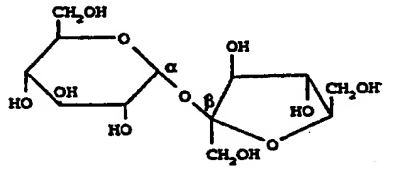


α,α'-trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside)



Sucrose (β-D-fructofuranosyl-(2→1)-α-D-glucopyanoside)

Fig. 1—Haworth structures of sucrose and trehalose.

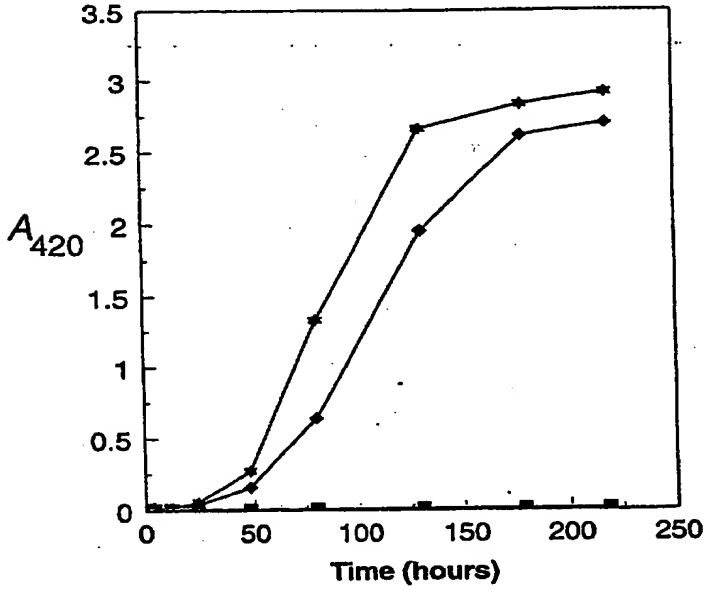


Fig. 2—Nonenzymatic browning (A_{420}) during storage at 40°C of model amorphous systems containing glucose (—★—), sucrose (—◆—) or trehalose (—■—).

12.0 hr at 90°C; trehalose samples treated at 90°C were withdrawn at 4, 6, 12, 24, 34, 48, 80, 130, 178, and 194 hr. Subsequently, samples were immediately diluted with 4 mL distilled water and refrigerated prior to analysis within 24 hr. Samples were diluted as appropriate prior to spectrophotometric analysis. The need to filter samples did not arise.

Nonenzymatic browning was monitored using the absorbance values at 420 nm and at 280 nm. The latter was used as an indicator of the formation of colorless intermediates with strongly absorbing carbonyl and other functions (e.g. hydroxymethylfurfural) (Feather, 1989). Absorbance measurements were conducted using a Perkin-Elmer Lambda 5 spectrophotometer.

Data analysis was conducted using the Minitab statistical package (Ryan et al., 1976) on an Amstrad 1640 PC. Zero, first and second order models were used to analyze data from A_{280} and A_{420} measurements. The most appropriate model was selected on the basis f visual assessment and the coefficient of determination (R^2) obtained from regression analysis. Rate constants were obtained from the best-fit regression equations. According to the method of Arabshahi and Lund (1985), data points from induction periods were excluded from regression analysis. Regression analysis was also employed to obtain values for activation energies.

RESULTS & DISCUSSION

DSC MEASUREMENTS on both the glucose (T_g = −28.5°C) and sucrose (T_g = −18.7°C) systems indicated that both systems were in the rubbery state for all temperatures studied. The presence of a large endothermic peak centered at 95°C in the trehalose system was interpreted as largely a consequence of crystal melting rather than a glass transition; the peak probably corresponds to the melting of trehalose dihydrate (m.p. 96.5–97.5°C). Since a baseline shift was not observed at any other region of the thermogram, it was likely that the glass transition for the amorphous phase of the trehalose system was obscured by the melting of the crystalline phase. The presence of crystalline trehalose in the system was confirmed using solid-state NMR spectroscopy. Analysis was based on chemical shift differences between amorphous and crystalline systems; spectra of amorphous systems resemble those of solution-state systems except that peaks are broader. A reference spectrum of trehalose in solution was used for comparison. Thus, the trehalose systems heated at 40°C and 60°C were mixed amorphous glass-crystalline systems. The trehalose system heated at 90°C was clear and rubbery. There was no evidence of crystallization in the sucrose or glucose systems.

Moisture contents of the equilibrated trehalose and sucrose systems were 7.4 and 5.9%, respectively. Moisture content of the glucose system was unexpectedly low (2.4%). The lower percentage of sugar in the glucose system may have contributed to its lower moisture content.

The browning of all systems at 40°C showed unexpected deviations from zero-order behavior late in the storage period (Fig. 2). However, the best fit model for the data in every case was zero order (Table 1) and therefore pseudo zero-order rate constants have been reported. The goodness of fit for the zero-order plots for A_{420} at 60°C and 90°C and for A_{280} at all temperatures were extremely high as judged visually and by regression analysis (Table 1).

Clearly, the rate of nonenzymatic browning in the trehalose system was much lower (Fig. 2, Table 2) than that in the sucrose or glucose systems. Rate constants for brown color formation (A_{420}) were of the order of ~200 to 2000-fold greater for the sucrose system than for the trehalose system, depending on temperature. Differences between A_{280} values for the two systems were even greater, possibly reflecting the high molar extinction coefficients of intermediates absorbing at 280 nm (e.g. 5-hydroxymethylfurfural). The rate of browning in the sucrose system was lower than in the glucose system with the exception of the system at 40°C where rate constants for brown color formation were similar (Table 2). Insoluble melanoidins formed in the glucose system heated at 90°C for 12 hr and, therefore, that time point was excluded from regression analysis. All other samples were transparent.

The presence of crystalline material in the trehalose system at 40 and 60°C may have influenced the overall rate of hydrolysis, stabilizing the system. However, at 90°C, all systems were in the rubbery state and there were substantial differences between the stability of the sucrose and trehalose system: 200-fold for A_{420} values and 895-fold for A_{280} values. The comparative susceptibility of the trehalose system to crystallization may be attributable to the symmetry of the trehalose molecule which may facilitate crystal formation and growth compared with the unsymmetrical sucrose molecule.

The browning curves of all systems showed evidence of an induction period (Fig. 2). This is generally expected of nonenzymatic browning reactions (Labuza, 1994), the extent of the induction period depending on reaction conditions and reflecting

Table 1—Coefficients of determination (R^2) for fits to zero, first or second order kinetic models for absorbance data collected at 280 nm or 420 nm[a]

	T (°C)	Reaction order	Glucose (R^2, %)	Sucrose (R^2, %)	Trehalose (R^2, %)
A_{280}	40°C	0	100	99.6	93.9
		1	88.9	88.6	91.0
		2	65.9	55.4	86.7
	60°C	0	97.8	98.6	97.6
		1	91.2	90.8	92.0
		2	82.2	62.1	82.3
	90°C	0	98.4	99.8	98.8
		1	49.7	79.4	85.9
		2	20.7	41.6	44.9
A_{420}	40°C	0	83.9	94.1	84.8
		1	69.9	82.5	79.0
		2	54.3	61.0	72.6
	60°C	0	96.0	94.1	93.5
		1	86.7	84.3	90.3
		2	58.8	48.3	73.8
	90°C	0	94.1	97.8	98.8
		1	79.6	75.3	92.2
		2	41.6	43.4	59.9

[a] Data ranges subjected to regression analysis as selected to exclude induction periods are reported in Table 2.

Table 2—Pseudo-zero order rate constants for nonenzymatic browning in freeze-dried sugar systems (a_w, 0.33) stored at 40, 60 or 90°C[a]

	T(°C)		Glucose	Sucrose	Trehalose
A_{280}	40	k_0 (AU·hr^{-1})	0.525 ±0.019	0.301 ± 0.023	0.000282 ± 0.00006
		time range	48-218 hr (n=5)	12-218 hr (n=7)	0-218 hr (n=9)
	60	k_0 (AU·hr^{-1})	5.95 ± 1.01	3.55 ± 0.48	0.00155 ± 0.00028
		time range	24-218 hr (n=7)	24-218 hr (n=7)	24-218 hr (n=7)
	90	k_0 (AU·hr^{-1})	380.0 ± 49.5	171.0 ± 8.8	0.191 ± 0.021
		time range	0-6 hr (n=8)	1-12 hr (n=7)	12-194 hr (n=8)
A_{420}	40	k_0 (AU·hr^{-1})	0.0153 ± 0.0123	0.0162 ± 0.0074	0.000020 ± 0.000007
		time range	48-218 hr (n=5)	48-218 hr (n=5)	0-218 hr (n=9)
	60	k_0 (AU·hr^{-1})	0.315 ± 0.073	0.238 ± 0.068	0.000131 ± 0.000025
		time range	24-218 hr (n=7)	24-218 hr (n=7)	0-218 hr (n=11)
	90	k_0 (AU·hr^{-1})	22.2 ± 6.3	10.1 ± 2.1	0.0459 ± 0.0060
		time range	0.5-6 hr (n=7)	1.5-12 hr (n=6)	24-194 hr (n=7)

[a] Data are means ± 95% confidence intervals. Time ranges for kinetic analysis were selected visually to exclude induction periods. AU, absorbance units.

Table 3—Activation energy for non-enzymatic browning of freeze-dried sugar systems stored over the range 40–90°C

	E_a (kcal.mol^{-1})	
Sugar	A_{280}	A_{420}
Glucose	29.9	33.0
Sucrose	28.7	29.2
Trehalose	29.9	35.6

the step-wise nature of the reaction via several colorless intermediates. The lengths of induction periods for nonenzymatic browning are usually inversely proportional to the rate of browning (Petriella et al., 1988). In addition to the formation of colorless intermediates from monosaccharide reactants, it is likely that the initial hydrolysis step contributed to the induction periods observed for the sucrose and trehalose systems. Even the production of colorless advanced intermediates as evidenced by the A_{280} curves showed induction periods. Data points for induction periods were excluded from kinetic analysis (Table 1).

The temperature dependence of reaction rates in dehydrated amorphous systems, such as those examined here, is complex. At low a_w values, the Maillard reaction is generally diffusion limited, especially if the system is glassy (Karel and Buera, 1994). Since the diffusivity depends on the viscosity, which in turn depends on temperature, temperature may exert a considerable influence on the mechanism as well as on the rate of reaction. The influence of temperature on the physical state is determined by the glass transition temperature (T_g). Browning rates below T_g are relatively low in most systems (Karmas et al., 1992; Roos and Himberg, 1994; Buera and Karel, 1995). The extreme structural changes that result from the glass transition have been associated with changes in activation energy values for nonenzymatic browning (Karmas et al., 1992). However, recent results suggest that the impact of the glass transition on sucrose hydrolysis may be less than previously suggested. Schebor et al. (1995) reported that sucrose hydrolysis occurred to a notable extent in a starch system in the glassy state. They concluded that the rate of sucrose hydrolysis in the starch glass system could not be predicted by T_g alone and emphasized the importance of factors such as moisture content, temperature, and especially moisture-dependent pH changes in predicting hydrolysis rates. Arrhenius plots for the glucose and sucrose systems showed good linear relationships (R^2 = 99.4–100%). However, the trehalose plots showed slight deviation from linearity (R^2 = 95.5–96.2%) which could be interpreted as a shift from a lower activation energy below 60°C to a higher one in the 60–90°C range. Thus, the activation energy values reported for browning of the trehalose system must be regarded as approximate.

The activation energy values (Table 3) are in the range normally expected for nonenzymatic browning reactions (16.7–43.0 kcal/mol^{-1})(O'Brien, 1995). The activation energies for formation of substances absorbing at 280 nm were similar to the value of 28.1 kcal/mol^{-1} reported for 5-HMF formation in a model system (Schirle-Keller and Reineccius, 1992). The trend in activation energy values for sucrose and trehalose systems was similar to that for activation energies reported for acid hydrolysis; a value of 26.3 kcal/mol^{-1} has been reported for sucrose hydrolysis in saturated solution (Schoebel et al., 1969), whereas a value of 40.2 kcal/mol^{-1} has been reported for trehalose hydrolysis (BeMiller, 1967).

The present experiments made no assumptions concerning the mechanism of browning. The protonation of the amino groups of lysine at the pH of the three systems would likely be complete, inhibiting the early stages of the Maillard reaction. Therefore, direct sugar degradation probably makes a significant contribution to the overall browning rate. Nevertheless, the presence of amino acid appears to have an important catalytic effect on the development of brown color as evidenced by a considerably lower rate of browning in the absence of lysine (O'Brien, J, unpublished results). It has been suggested that the presence of amino acids may promote sugar degradation by catalyzing Lobrey de Bruyn-Alberda van Ekenstein transformations and by increasing the mutarotation rates of sugars (Shallenberger, 1984).

Although the rate of browning in the sucrose system was high, it may be concluded that hydrolysis was a significant rate-limiting factor (since the rate of browning in the glucose system was greater). Complete hydrolysis of sucrose would result in double the molar concentration of monosaccharides available for reaction compared with the glucose system. The extremely low rate of browning in the trehalose system may be attributable largely to the rate limiting hydrolysis step although it is likely that differences in physical properties also contributed, especially at 40 and 60°C. In addition, it is likely that the greater reactivity of fructose compared with glucose may have contributed to the higher rate of browning in the sucrose system. Bunn and Higgins (1981) showed that the formation of Schiff's base (the first step in the Maillard reaction) from amino groups of hemoglobin in a reaction involving fructose was 7.5 times faster than a reaction where the reacting sugar was glucose.

Results suggest that freeze-dried systems formed from trehalose were exceedingly stable to nonenzymatic browning in the

presence of lysine compared with systems containing sucrose. Therefore, use of trehalose probably could contribute to the stabilization of systems in which nonenzymatic browning is a major quality-limiting factor.

Further studies are needed to determine the kinetics and molecular mechanisms of acid hydrolysis of sucrose and trehalose under these conditions.

REFERENCES

Arabshahi, A. and Lund, D.B. 1985. Considerations in calculating kinetic parameters from experimental data. J. Food Process Eng. 7: 239–251.

Bell, L.N. and Labuza, T.P. 1992. Compositional influence on the pH of reduced-moisture solutions. J. Food Sci. 57: 732–734.

BeMiller, J.N. 1967. Acid-catalyzed hydrolysis of glycosides, Adv. Carbohydr. Chem. 22: 25–108.

Brown, M.R., Keith, T.J. and Knull, H.R. 1990. Decreased actin activated myosin ATPase activity by non-enzymatic glycation, In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, and R. Liardon (Ed.), p. 487–492. Birkhäuser Verlag, Basel, Switzerland.

Buera, M.P. and Karel, M.P. 1995. Effect of physical changes on the rates of nonenzymatic browning and related reactions. Food Chem. 52: 167–173.

Bunn, H.F. and Higgins, P.J. 1981. Reaction of monosaccharides with proteins: possible evolutionary significance. Science 213: 222–224.

Burke, M.J. 1986. The glassy state and survival of anhydrous biological systems, In *Membranes, Metabolism and Dry Organisms*, A.C. Leopold, (Ed.), p. 358–363. Comstock Publishing Associates, Ithaca, NY.

Cerami, A. 1994. The role of the Maillard reaction *in vivo*, in *Maillard Reactions in Chemistry, Food, and Health*, T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brien and J.W. Baynes (Ed.), p.1–10. Royal Society of Chemistry, Cambridge, UK.

Crowe, J.H., Crowe, L.M., and Mouradian, R. 1983. Stabilization of biological membranes at low water activities. Cryobiology 20: 346–356.

Feather, M.S. 1989. The formation of deoxyglycosuloses (deoxyosones) and their reactions during the processing of food, in *Frontiers in Carbohydrate Research-1*, R.P. Millane, J.N. BeMiller, and R. Chandrasekaran (Ed.), p. 66–73. Elsevier Science Publishers, New York.

Flink, J.M. 1983. Nonenzymatic browning of freeze-dried sucrose. J. Food Sci. 48: 539–542.

Fox, K.C. 1995. Putting proteins under glass. Science 267: 1922–1923.

Franks, F. 1994. Long-term stabilization of biomolecules. Bio/Technology 12: 253–256.

Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Natl. Bur. Stand. Phys. Chem. 81A: 89–96.

Karel, M. and Buera, M.P. 1994. Glass transition and its potential effects on kinetics of condensation reactions and in particular on nonenzymatic browning, in *Maillard Reactions in Chemistry, Food, and Health*, T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brien, and J.W. Baynes (Ed.), p. 164–169. Royal Society of Chemistry, Cambridge, UK.

Karel, M. and Labuza, T.P. 1968. Nonenzymatic browning in model systems containing sucrose. J. Agric. Food Chem. 16: 717–719.

Karmas, R., Buera, M.P., and Karel, M. 1992. Effect of glass transition on rates of nonenzymatic browning in food systems. J. Agric. Food Chem. 40: 873–879.

Labuza, T.P. 1994. Interpreting the complexity of the kinetics of the Maillard reaction, in *Maillard Reactions in Chemistry, Food, and Health*, T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brien, and J.W. Baynes (Ed.), p. 176–181. Royal Society of Chemistry, Cambridge, UK.

Lee, H.S. and Nagy, S. 1990. Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. J. Food Proc. Preserv. 14: 171–178.

O'Brien, J. 1995. Heat-induced changes in lactose: isomerization, degradation, Maillard browning, in *Heat-Induced Changes in Milk*, P.F. Fox, (Ed.), p. 134–170. International Dairy Federation, Brussels, Belgium.

O'Brien, J. and Morrissey, P.A. 1989. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit. Rev. Food Sci. Nutr. 28: 211–248.

Panek, A.C., Bernardes, E., and Panek, A.D. 1986. Does trehalose play a role in yeast cells under stress? in *Membranes, Metabolism, and Dry Organisms*, A.C. Leopold (Ed.), p. 123–142. Comstock Publishing Associates, Ithaca, NY.

Petriella, C., Chirife, J., Resnik, S.L., and Lozano, R.D. 1988. Correlation between induction time and rate of browning in heated model solutions of glucose and lysine. Int. J. Food Sci. Technol. 23: 415–418.

Roos, Y.H. and Himberg, M-J. 1994. Nonenzymatic browning behaviour, as related to glass transition, of a food model at chilling temperatures. J. Agric. Food Chem. 42: 893–898.

Roos, Y. and Karel, M. 1991. Phase transitions of mixtures of amorphous polysaccharides and sugars. Biotechnol. Prog. 7: 49–53.

Roser, B. 1991. Trehalose, a new approach to premium dried foods. Trends Food Sci. Technol. 2: 166–169.

Ryan, T.A., Joiner, B.L., and Ryan, B.F. 1976. *Minitab Student Handbook*. Duxbury Press, Boston.

Sapru, V. and Labuza, T.P. 1993. Glassy state in bacterial spores predicted by polymer glass-transition theory. J. Food Sci. 58: 445–448.

Schebor, C., Buera, M.P., Chirife, J., and Karel, M. 1995. Sucrose hydrolysis in a glassy starch matrix, Lebensm. Wiss. Technol. 28: 245–248.

Schirle-Keller, J.P. and Reineccius, G.A. 1992. Reaction kinetics for the formation of oxygen-containing heterocyclic compounds in model systems, in *Flavour Precursors. Thermal and Enzymatic Conversions*, R. Teranishi G.R. Takeoka, and M. Guntert (Ed.), p. 244–258. American Chemical Society, Washington, DC.

Schoebel, T., Tannenbaum, S.R., and Labuza, T.P. 1969. Reaction at limited water concentration. I. Sucrose hydrolysis. J. Food Sci. 34: 324–329.

Shallenberger, R.S. 1984. Nature of the amino acid catalysis of the glucose mutarotation reaction, Food Chem. 15: 1–7.

Van Boekel, M. and Hoenders, H. 1990. *In vitro* and *in vivo* glycation of human IgG, in *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, and R. Liardon (Ed.), p. 431–436. Birkhäuser Verlag, Basel, Switzerland.

Wasylyk, J.M., Tice, A.R., and Baust, J.G. 1988. Partial glass formation: a novel mechanism of insect cryoprotection. Cryobiology 25: 451–458.

Williams, R.J. and Leopold, A.C. 1989. The glassy state in corn embryos Plant Physiol. 89: 977–981.

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REFERENCES

Banga, J.R., Perez-Martin, R.I., Gallardo, J.M., and Casares, J.J. 1991. Optimization of the processing of conduction-heated canned foods: Study of several objective functions. J. Food Eng. 14: 25.

Holdsworth, S.D. 1985. Optimization of thermal processing—A review. J. Food Eng. 4: 89.

Kano, H. 1987. *Optimal Control Theory of System and Optimization*. Corona Co. Tokyo, Japan.

Kato, K. 1988. *Optimal Control in Engineering*. Tokyo University Press. Tokyo, Japan.

Lasdon, L.S., Mitter, S.K., and Waren, A.D. 1967. The conjugate gradient method for optimal control problems. IEEE Trans. on Automatic Control 12(2): 132.

Lund, D.B. 1982. Applications of optimization in heat processing. Food Technol. 36(7): 97.

Mansfield, T. 1962. High temperature-short time sterilization. Proc. of 1st Int. Congress on Food Sci & Technol., London, U.K. 4: 311.

Nadkarni, M. and Hatton, T. 1985. Optimal nutrient retention during the thermal processing of conduction-heated canned foods: Application of the distributed minimum principle. J. Food Sci. 50: 1312.

Ohlsson, T. 1980a. Temperature dependence of sensory quality changes during thermal processing. J. Food Sci. 45: 836.

Ohlsson, T. 1980b. Optimal sterilization temperatures for flat containers. J. Food Sci. 45: 848.

Quintana, V.H. and Davison, E.J. 1973. A numerical method for solving optimal problems with unspecified terminal time. Int. J. Control 17(1): 97.

Saguy, I. and Karel, M. 1979. Optimal retort temperature profile in optimizing thiamine retention in conduction type heating of canned foods. J. Food Sci. 44: 1485.

Silva, C.L.M., Oliveira, F.A.R. and Hendrickx, M. 1993. Modelling optimum processing conditions for the sterilization of prepackaged foods. Food Control 4(2): 67.

Teixeira, A.A., Dixon, J.R., Zahradnik, J.W. and Zinsmeister, G.E. 1969. Computer optimization of nutrient retention in the thermal processing of conduction-heated foods. Food Technol. 23(6): 137.

Teixeira, A.A., Zinsmeister, G.E. and Zahradnik, J.W. 1975. Computer simulation of variable retort control and container geometry as a possible means of improving thiamine retention in thermally processed foods. J. Food Sci. 40: 656.

Thijssen, H.A.C., Kerkhof, P.J.A.M. and Liefkens, A.A.A. 1978. Short-cut method for the calculations of sterilization conditions yielding optimum quality retention for conduction-type heating of packaged foods. J. Food Sci. 43: 1096.

Yamaguchi, K. and Kishimoto, A. 1976. In-package high-temperature short-time sterilization of foods packaged in retortable pouches. Presented at International Conference of Prevention of Spoilage through Packaging, Munich, Germany.

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